

WHAT IS CLAIMED IS:

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1. A method of cultivating a mammalian cell in suspension *in vitro*, comprising:  
(a) obtaining a mammalian cell to be cultivated in suspension;  
and  
(b) contacting said cell with a serum-free cell culture medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension.

2. The method of claim 1, wherein said polyanionic compound is a polysulfonated compound or a polysulfated compound.

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3. The method of claim 2, wherein said polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

4. The method of claim 2, wherein said polysulfonated or polysulfated compound is dextran sulfate.

5. The method of claim 4, wherein said dextran sulfate has an average molecular weight of about 5,000 daltons.

6. The method of claim 4, wherein said medium is protein-free.

7. The method of claim 1, wherein said medium is a 1X medium formulation.

8. The method of claim 1, wherein said medium formulation is a 10X concentrated medium formulation.

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9. The method of claim 1, wherein said medium further comprises one or more ingredients selected from the group of ingredients consisting of one or more amino acids, one or more vitamins, one or more inorganic salts, one or more buffering salts, one or more sugars, one or more lipids, transferrin (or transferrin substitute), and insulin (or insulin substitute).

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10. The method of claim 9, wherein said medium further comprises one or more supplements selected from the group of supplements consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

11. The method of claim 10, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

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12. The method of claim 9, wherein said amino acid ingredient comprises one or more amino acids selected from the group consisting of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine.

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13. The method of claim 9, wherein said vitamin ingredient comprises one or more vitamins selected from the group consisting of biotin, choline chloride, D-Ca<sup>++</sup>-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine and vitamin B<sub>12</sub>.

14. The method of claim 9, wherein said inorganic salt ingredient comprises one or more inorganic salts selected from the group consisting of one or more calcium salts,  $\text{Fe}(\text{NO}_3)_3$ , KCl, one or more magnesium salts, one or more manganese salts, NaCl,  $\text{NaHCO}_3$ ,  $\text{Na}_2\text{HPO}_4$ , one or more selenium salts, one or more vanadium salts and one or more zinc salts.

15. A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension;  
and

(b) contacting said cell with a cell culture medium comprising the ingredients ethanolamine, D-glucose, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D- $\text{Ca}^{++}$ -pantothenate, folic acid, *D*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin  $\text{B}_{12}$ , at least one polyanionic or polycationic compound, one or more calcium salts, KCl, one or more iron salts, one or more magnesium salts, one or more manganese salts, NaCl,  $\text{NaHCO}_3$ ,  $\text{Na}_2\text{HPO}_4$ , one or more selenium salts, one or more vanadium salts and one or more zinc salts, wherein each ingredient is present in an amount which supports the cultivation of said cell in suspension.

16. The method of claim 15, wherein said polyanionic compound is a polysulfonated or polysulfated compound.

17. The method of claim 16, wherein said polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

18. The method of claim 17, wherein said polysulfonated or polysulfated compound is dextran sulfate.

19. The method of claim 18, wherein said dextran sulfate has an average molecular weight of about 5,000 daltons.

20. The method of claim 15, wherein said medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

21. The method of claim 20, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

22. A method of cultivating a mammalian cell in suspension *in vitro*, comprising:  
(a) obtaining a mammalian cell to be cultivated in suspension;  
and  
(b) contacting said cell with a cell culture medium obtained by combining a basal medium with at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension.

23. The method of claim 22, wherein said polyanionic compound is a polysulfonated or polysulfated compound.

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5 24. The method of claim 23, wherein said polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

25. The method of claim 23, wherein said polysulfonated or polysulfated compound is dextran sulfate.

10 26. The method of claim 25, wherein said dextran sulfate has an average molecular weight of about 5,000 daltons.

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15 27. The method of claim 22, wherein said basal medium is obtained by combining one or more ingredients selected from the group consisting of ethanolamine, D-glucose, N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca<sup>++</sup>-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, 20 thiamine, vitamin B<sub>12</sub>, one or more calcium salts, one or more iron salts, KCl, one or more magnesium salts, one or more manganese salts, NaCl, NaHCO<sub>3</sub>, Na<sub>2</sub>HPO<sub>4</sub>, one or more selenium salts, one or more vanadium salts and one or more zinc salts,

25 wherein each ingredient is added in an amount which supports the cultivation of said cell in suspension.

28. The method of claim 22, wherein said medium is obtained by combining said basal medium and one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

29. The method of claim 28, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

30. The method of any one of claims 1, 15 or 22, wherein said mammalian cell is a mammalian epithelial cell.

31. The method of claim 30, wherein said mammalian epithelial cell is selected from the group consisting of a keratinocyte, a cervical epithelial cell, a bronchial epithelial cell, a tracheal epithelial cell, a kidney epithelial cell and a retinal epithelial cell.

32. The method of claim 30, wherein said cell is a human cell.

33. The method of claim 32, wherein said human cell is a 293 embryonic kidney cell, a HeLa cervical epithelial cell, a PER-C6 retinal cell, or a derivative thereof.

34. The method of claim 33, wherein said human cell is a 293 embryonic kidney cell.

35. The method of claim 30, wherein said cell is a normal cell.

36. The method of claim 30, wherein said cell is an abnormal cell.

37. The method of claim 36, wherein said abnormal cell is a transformed cell, an established cell, or a cell derived from a diseased tissue sample.

38. A kit for the cultivation of a mammalian epithelial cell in suspension *in vitro*, said kit comprising one or more containers, wherein a first container contains a serum-free culture medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension.

39. A kit for the cultivation of a mammalian epithelial cell in suspension *in vitro*, said kit comprising one or more containers, wherein a first container contains a cell culture medium comprising the ingredients ethanolamine, D-glucose, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca<sup>++</sup>-pantothenate, folic acid, *D*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B<sub>12</sub>, one or more calcium salts, KCl, one or more iron salts, one or more magnesium salts, one or more manganese salts, NaCl, NaHCO<sub>3</sub>, Na<sub>2</sub>HPO<sub>4</sub>, one or more selenium salts, one or more vanadium salts and one or more zinc salts,

wherein each ingredient is present in an amount which supports the cultivation of said cell in suspension.

40. The kit of claim 39, wherein said culture medium in said first container further comprises at least one polyanionic or polycationic compound.



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46. The kit of claim 38 or claim 39, further comprising one or more additional containers containing one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

47. The kit of claim 46, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

48. A composition comprising a serum-free culture medium comprising at least one polyanionic or polycationic compound, wherein said culture medium supports the cultivation of a mammalian cell in suspension *in vitro*.



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53. The composition of claim 48, further comprising at least one virus.

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57. The composition of claim 55, wherein said cell is a human cell.

58. The composition of claim 57, wherein said human cell is a 293 embryonic kidney cell, a HeLa cervical epithelial cell, a PER-C6 retinal cell or a derivative thereof.

59. The composition of claim 55, wherein said cell is a normal cell.

60. The composition of claim 55, wherein said cell is an abnormal cell.

61. The composition of claim 60, wherein said abnormal cell is a transformed cell, an established cell, or a cell derived from a diseased tissue sample.

62. A composition for use in suspension cultivation of a mammalian cell, comprising a serum-free culture medium and at least one polyanionic or polycationic compound.

63. The composition of claim 62, wherein said polyanionic compound is a polysulfonated or polysulfated compound.

64. The composition of claim 63, wherein said polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

65. The composition of claim 63, wherein said polysulfonated or polysulfated compound is dextran sulfate.

66. The composition of claim 65, wherein said dextran sulfate has an average molecular weight of about 5,000 daltons.

67. A serum-free culture medium for use in suspension cultivation of a mammalian cell, comprising at least one polyanionic or polycationic compound.

68. A serum-free culture medium for use in producing a virus, said medium comprising at least one polyanionic or polycationic compound, wherein a virus-infected mammalian cell cultivated in suspension in said medium produces a higher virus titer than a mammalian cell not cultivated in suspension in said medium.

69. The culture medium of claim 67 or claim 68, wherein said polyanionic compound is a polysulfonated or polysulfated compound.

70. The culture medium of claim 69, wherein said polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

71. The culture medium of claim 69, wherein said polysulfonated or polysulfated compound is dextran sulfate.

72. The culture medium of claim 71, wherein said dextran sulfate has an average molecular weight of about 5,000 daltons.

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73. A method of producing a virus comprising  
(a) obtaining a mammalian cell to be infected with a virus;  
(b) contacting said cell with a virus under conditions suitable to promote the infection of said cell by said virus; and

5 (c) cultivating said cell according to the method of any one of claims 1, 15 or 22, under conditions suitable to promote the production of said virus by said cell.

74. The method of claim 73, wherein said mammalian cell is an epithelial cell.

10 75. The method of claim 73, wherein said mammalian cell is a human cell.

76. The method of claim 75, wherein said human cell is a 293 embryonic kidney cell.

15 77. The method of claim 75, wherein said virus is an adenovirus, an adeno-associated virus or a retrovirus.

~~78. A virus produced according to the method of claim 73.~~

79. A method of producing a polypeptide comprising  
(a) obtaining a mammalian cell that has been genetically engineered to produce a polypeptide; and

20 (b) cultivating said cell according to the method of any one of claims 1, 15 or 22, under conditions favoring expression of said polypeptide by said mammalian cell.

80. The method of claim 79, wherein said mammalian cell is an epithelial cell.

81. The method of claim 79, wherein said mammalian cell is a human cell.

82. The method of claim 81, wherein said human cell is a 293 embryonic kidney epithelial cell.

~~83. A polypeptide produced according to the method of claim 79.~~

84. A eukaryotic cell culture medium comprising a  $\text{Fe}^{2+}$  chelate and a  $\text{Zn}^{2+}$  salt, wherein said medium is capable of supporting the high-density growth of mammalian cells in suspension culture and/or the expression of recombinant protein.

85. The eukaryotic cell culture medium according to claim 84, wherein said medium contains neither transferrin nor insulin.

86. The eukaryotic cell culture medium according to claim 84, wherein said mammalian cells are Chinese hamster ovary cells.

87. The eukaryotic cell culture medium according to claim 84, wherein said medium is a 1X medium formulation.

88. The eukaryotic cell culture medium according to claim 84, wherein said medium is a concentrated medium formulation.

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95. The eukaryotic cell culture medium according to claim 94, wherein said polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

96. The eukaryotic cell culture medium according to claim 95, wherein said polysulfonated or polysulfated compound is dextran sulfate.

97. The eukaryotic cell culture medium according to claim 96, wherein said dextran sulfate has an average molecular weight of 5,000 daltons.

5 98. The eukaryotic cell culture medium according to claim 84 or 93, further comprising one or more ingredients selected from the group consisting of L-arginine, L-asparagine·H<sub>2</sub>O, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-lysine·HCl, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, L-cystine·2HCl, Na<sub>2</sub>HPO<sub>4</sub>, pyridoxine·HCl, thiamine·HCl, glutathione, cupric sulfate·7H<sub>2</sub>O, cadmium chloride·5H<sub>2</sub>O, cobalt chloride·2H<sub>2</sub>O, stannous chloride·2H<sub>2</sub>O, manganous sulfate·H<sub>2</sub>O, nickel sulfate·6H<sub>2</sub>O, sodium metavanadate, ammonium molybdate·4H<sub>2</sub>O, barium acetate, potassium bromide, potassium iodide, chromium sulfate, sodium fluoride, silver nitrate, rubidium chloride, zirconyl chloride, aluminum chloride, germanium dioxide, titanium tetrachloride, sodium metasilicate, magnesium chloride (anhydrous), D-calcium pantothenate, calcium nitrate·4H<sub>2</sub>O, potassium chloride, ascorbic acid magnesium salt phosphate, pluronic F68 10% solution, Na<sub>2</sub>HPO<sub>4</sub>, D-glucose, folic acid, riboflavin, biotin, choline chloride, niacinamide, i-inositol, sodium pyruvate, vitamin B-12, β-mercaptoethanol, para-aminobenzoic acid, β-glycerophosphate, sodium selenite, ethanolamine·HCl, spermine, putrescine·2HCl, monothioglycerol, and sodium bicarbonate,

20 wherein each of said ingredients is present in an amount which supports the high-density growth of Chinese hamster ovary cells in suspension culture and/or the expression of recombinant protein.

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5 99. A eukaryotic cell culture medium obtained by combining ferrous sulfate·EDTA and  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , together with a eukaryotic medium, wherein said ferrous sulfate·EDTA and said  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  are each present in an amount which supports the high-density growth of Chinese hamster ovary cells in suspension culture.

100. The eukaryotic cell culture medium according to claim 99, further comprising a polyanionic or polycationic compound, wherein said polyanionic or polycationic compound is present in an amount sufficient to prevent cell clumping and/or increase the level of recombinant protein expression.

10 101. The eukaryotic cell culture medium according to claim 100, wherein said polyanionic compound is a polysulfonated compound or a polysulfated compound.

15 102. The eukaryotic cell culture medium according to claim 101, wherein said polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

103. The eukaryotic cell culture medium according to claim 102, wherein said polysulfonated or polysulfated compound is dextran sulfate.

20 104. The eukaryotic cell culture medium according to claim 103, wherein said dextran sulfate has an average molecular weight of 5,000 daltons.

105. The eukaryotic cell culture medium obtained according to claim 99 or 100, further comprising one or more ingredients selected from the group consisting of L-arginine, L-asparagine· $\text{H}_2\text{O}$ , L-aspartic acid, L-glutamic acid, L-

histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-lysine·HCl, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, L-cystine·2HCl, Na<sub>2</sub>HPO<sub>4</sub>, pyridoxine·HCl, thiamine·HCl, glutathione, cupric sulfate·5H<sub>2</sub>O, cadmium chloride·5H<sub>2</sub>O, cobalt chloride·2H<sub>2</sub>O, stannous chloride·2H<sub>2</sub>O, manganous sulfate·H<sub>2</sub>O, nickel sulfate·6H<sub>2</sub>O, sodium metavanadate, ammonium molybdate·4H<sub>2</sub>O, barium acetate, potassium bromide, potassium iodide, chromium sulfate, sodium fluoride, silver nitrate, rubidium chloride, zirconyl chloride, aluminum chloride, germanium dioxide, titanium tetrachloride, sodium metasilicate, magnesium chloride (anhydrous), D-calcium pantothenate, calcium nitrate·4H<sub>2</sub>O, potassium chloride, ascorbic acid magnesium salt phosphate, pluronic F68 10% solution, Na<sub>2</sub>HPO<sub>4</sub>, D-glucose, folic acid, riboflavin, biotin, choline chloride, niacinamide, i-inositol, sodium pyruvate, vitamin B-12, β-mercaptoethanol, para-aminobenzoic acid, β-glycerophosphate, sodium selenite, ethanolamine·HCl, spermine, putrescine·2HCl, monothioglycerol, and sodium bicarbonate,

wherein each of said ingredients is present in an amount which supports the high-density growth of Chinese hamster ovary cells in suspension culture and/or the expression of recombinant protein.

106. A method of cultivating mammalian cells in suspension culture to high density and/or expressing a recombinant protein, said method comprising the steps of

(a) contacting said cells with the eukaryotic cell culture medium of claim 84, wherein said Fe<sup>2+</sup> chelate and said Zn<sup>2+</sup> salt are each present in an amount which supports the growth of mammalian cells in culture; and

(b) cultivating said mammalian cells under conditions suitable to support the growth of said cells to high density and/or the expression of said recombinant protein.

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107. The method according to claim 106, further comprising a polyanionic or polycationic compound, wherein said polyanionic or polycationic compound is present in an amount sufficient to prevent cell clumping and/or increase the level of recombinant protein expression.

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108. The method according to claim 107, wherein said polyanionic compound is a polysulfonated compound or a polysulfated compound.

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109. The method according to claim 108, wherein said polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

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110. The method according to claim 109, wherein said polysulfonated or polysulfated compound is dextran sulfate.

111. The method according to claim 110, wherein said dextran sulfate has an average molecular weight of 5,000 daltons.

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112. The method according to claim 106 or 107, wherein said eukaryotic cell culture medium further comprises one or more ingredients selected from the group consisting of L-arginine, L-asparagine·H<sub>2</sub>O, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-lysine·HCl, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, L-cystine·2HCl, Na<sub>2</sub>HPO<sub>4</sub>, pyridoxine·HCl, thiamine·HCl, glutathione, cupric sulfate·5H<sub>2</sub>O, cadmium chloride·5H<sub>2</sub>O, cobalt chloride·2H<sub>2</sub>O, stannous chloride·2H<sub>2</sub>O, manganous sulfate·H<sub>2</sub>O, nickel sulfate·6H<sub>2</sub>O, sodium metavanadate, ammonium molybdate·4H<sub>2</sub>O, barium acetate, potassium bromide, potassium iodide, chromium sulfate, sodium fluoride,

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silver nitrate, rubidium chloride, zirconyl chloride, aluminum chloride, germanium dioxide, titanium tetrachloride, sodium metasilicate, magnesium chloride (anhydrous), D-calcium pantothenate, calcium nitrate·4H<sub>2</sub>O, potassium chloride, ascorbic acid magnesium salt phosphate, pluronic F68 10% solution, Na<sub>2</sub>HPO<sub>4</sub>, D-glucose, folic acid, riboflavin, biotin, choline chloride, niacinamide, i-inositol, sodium pyruvate, vitamin B-12, β-mercaptoethanol, para-aminobenzoic acid, β-glycerophosphate, sodium selenite, ethanolamine·HCl, spermine, putrescine·2HCl, monothioglycerol, and sodium bicarbonate,

wherein each of said ingredients is present in an amount which supports the ~~high-density~~ growth of Chinese hamster ovary cells in suspension culture and/or the expression of recombinant protein.

113. An improved eukaryotic cell culture medium for growing Chinese hamster ovary cells in suspension culture to high density, the improvement comprising replacing insulin with a Zn<sup>2+</sup> salt and/or replacing transferrin with a Fe<sup>2+</sup> chelate and/or replacing transferrin with a Fe<sup>3+</sup> chelate.

114. The eukaryotic cell culture medium according to claim 113, further comprising a polyanionic or polycationic compound, wherein said polyanionic or polycationic compound is present in an amount sufficient to prevent cell clumping and/or increase the level of recombinant protein expression.

115. The eukaryotic cell culture medium according to claim 114, wherein said polyanionic compound is a polysulfonated compound or a polysulfated compound.

116. The eukaryotic cell culture medium according to claim 115, wherein said polysulfonated or polysulfated compound is selected from the group

consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

117. The eukaryotic cell culture medium according to claim 116, wherein said polysulfonated or polysulfated compound is dextran sulfate.

118. The eukaryotic cell culture medium according to claim 117, wherein said dextran sulfate has an average molecular weight of 5,000 daltons.

119. The eukaryotic cell culture medium according to claim 113, wherein the concentration of said  $\text{Fe}^{2+}$  or  $\text{Fe}^{3+}$ , if present, is about 0.00028 to 0.011 g/L and said concentration of said  $\text{Zn}^{2+}$ , if present, is about 0.00007 to 0.00073 g/L.

120. The eukaryotic cell culture medium according to claim 119, wherein said concentration of said  $\text{Fe}^{2+}$  or  $\text{Fe}^{3+}$ , if present, is about 0.0011 g/L and said concentration of said  $\text{Zn}^{2+}$ , if present, is about 0.000354 g/L.

121. The eukaryotic cell culture medium according to claim 113 or 114, further comprising one or more ingredients selected from the group consisting of L-arginine, L-asparagine· $\text{H}_2\text{O}$ , L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-lysine·HCl, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, L-cystine·2HCl,  $\text{Na}_2\text{HPO}_4$ , pyridoxine·HCl, thiamine·HCl, glutathione, cupric sulfate· $5\text{H}_2\text{O}$ , cadmium chloride· $5\text{H}_2\text{O}$ , cobalt chloride· $2\text{H}_2\text{O}$ , stannous chloride· $2\text{H}_2\text{O}$ , manganous sulfate· $\text{H}_2\text{O}$ , nickel sulfate· $6\text{H}_2\text{O}$ , sodium metavanadate, ammonium molybdate· $4\text{H}_2\text{O}$ , barium acetate, potassium bromide, potassium iodide, chromium sulfate, sodium fluoride, silver nitrate, rubidium chloride, zirconyl chloride, aluminum chloride, germanium dioxide, titanium

5 tetrachloride, sodium metasilicate, magnesium chloride (anhydrous), D-calcium pantothenate, calcium nitrate·4H<sub>2</sub>O, potassium chloride, ascorbic acid magnesium salt phosphate, pluronic F68 10% solution, Na<sub>2</sub>HPO<sub>4</sub>, D-glucose, folic acid, riboflavin, biotin, choline chloride, niacinamide, i-inositol, sodium pyruvate, vitamin B-12, β-mercaptoethanol, para-aminobenzoic acid, β-glycerophosphate, sodium selenite, ethanolamine·HCl, spermine, putrescine·2HCl, monothioglycerol, and sodium bicarbonate,

wherein each of said ingredients is present in an amount which supports the high-density growth of Chinese hamster ovary cells in suspension culture and/or the expression of recombinant protein.

122. An improved method of cultivating mammalian cells in suspension culture to high density, said improvement comprising the steps of

(a) contacting said cells with the eukaryotic cell culture medium of claim 84, wherein said Fe<sup>2+</sup> chelate and said Zn<sup>2+</sup> salt are each present in an amount which supports the high-density growth of mammalian cells in culture; and

(b) cultivating said mammalian cells under conditions suitable to support the high-density growth of said cells.

123. The method according to claim 122, further comprising a polyanionic or polycationic compound, wherein said polyanionic or polycationic compound is present in an amount sufficient to prevent cell clumping and/or increase the level of recombinant protein expression.

124. The method according to claim 123, wherein said polyanionic compound is a polysulfonated compound or a polysulfated compound.

125. The method according to claim 124, wherein said polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

126. The method according to claim 125, wherein said polysulfonated or polysulfated compound is dextran sulfate.

127. The method according to claim 126, wherein said dextran sulfate has an average molecular weight of 5,000 daltons.

128. The method according to claim 122 or 123, wherein said eukaryotic cell culture medium further comprises one or more ingredients selected from the group consisting of L-arginine, L-asparagine·H<sub>2</sub>O, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-lysine·HCl, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, L-cystine·2HCl, Na<sub>2</sub>HPO<sub>4</sub>, pyridoxine·HCl, thiamine·HCl, glutathione, cupric sulfate·5H<sub>2</sub>O, cadmium chloride·5H<sub>2</sub>O, cobalt chloride·2H<sub>2</sub>O, stannous chloride·2H<sub>2</sub>O, manganous sulfate·H<sub>2</sub>O, nickel sulfate·6H<sub>2</sub>O, sodium metavanadate, ammonium molybdate·4H<sub>2</sub>O, barium acetate, potassium bromide, potassium iodide, chromium sulfate, sodium fluoride, silver nitrate, rubidium chloride, zirconyl chloride, aluminum chloride, germanium dioxide, titanium tetrachloride, sodium metasilicate, magnesium chloride (anhydrous), D-calcium pantothenate, calcium nitrate·4H<sub>2</sub>O, potassium chloride, ascorbic acid magnesium salt phosphate, pluronic F68 10% solution, Na<sub>2</sub>HPO<sub>4</sub>, D-glucose, folic acid, riboflavin, biotin, choline chloride, niacinamide, i-inositol, sodium pyruvate, vitamin B-12, β-mercaptoethanol, para-aminobenzoic acid, β-glycerophosphate, sodium selenite, ethanolamine·HCl, spermine, putrescine·2HCl, monothioglycerol, and sodium bicarbonate,



wherein each of said ingredients is present in an amount which supports the high-density growth of Chinese hamster ovary cells in suspension culture.

129. A kit for the cultivation of a mammalian epithelial cell in suspension *in vitro*, said kit comprising one or more containers, wherein a first container contains a medium comprising a  $\text{Fe}^{2+}$  chelate and a  $\text{Zn}^{2+}$  salt, wherein said medium is capable of supporting the high-density growth of mammalian cells in suspension culture and/or the expression of recombinant protein.

130. The kit according to claim 129, wherein said medium contains neither transferrin nor insulin.

131. The kit according to claim 130, wherein said mammalian cells are Chinese hamster ovary cells.

132. The kit according to claim 129, said first container further comprising one or more ingredients selected from the group consisting of L-arginine, L-asparagine· $\text{H}_2\text{O}$ , L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-lysine· $\text{HCl}$ , L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, L-cystine· $2\text{HCl}$ ,  $\text{Na}_2\text{HPO}_4$ , pyridoxine· $\text{HCl}$ , thiamine· $\text{HCl}$ , glutathione, cupric sulfate· $7\text{H}_2\text{O}$ , cadmium chloride· $5\text{H}_2\text{O}$ , cobalt chloride· $2\text{H}_2\text{O}$ , stannous chloride· $2\text{H}_2\text{O}$ , manganous sulfate· $\text{H}_2\text{O}$ , nickel sulfate· $6\text{H}_2\text{O}$ , sodium metavanadate, ammonium molybdate· $4\text{H}_2\text{O}$ , barium acetate, potassium bromide, potassium iodide, chromium sulfate, sodium fluoride, silver nitrate, rubidium chloride, zirconyl chloride, aluminum chloride, germanium dioxide, titanium tetrachloride, sodium metasilicate, magnesium chloride (anhydrous), D-calcium pantothenate, calcium nitrate· $4\text{H}_2\text{O}$ , potassium chloride, ascorbic acid magnesium salt phosphate, pluronic F68 10% solution,  $\text{Na}_2\text{HPO}_4$ , D-glucose, folic acid,

riboflavin, biotin, choline chloride, niacinamide, i-inositol, sodium pyruvate, vitamin B-12,  $\beta$ -mercaptoethanol, para-aminobenzoic acid,  $\beta$ -glycerophosphate, sodium selenite, ethanolamine·HCl, spermine, putrescine·2HCl, monothioglycerol, and sodium bicarbonate,

5            wherein each of said ingredients is present in an amount which supports the high-density growth of Chinese hamster ovary cells in suspension culture and/or the expression of recombinant protein.

133.    The kit according to claim 132, wherein said medium in said first container further comprises at least one polyanionic or polycationic compound.

10            134.    The kit according to claim 132, further comprising a second container containing at least one polyanionic or polycationic compound.

135.    The kit according to any one of claims 132, 133, or 134, wherein said polyanionic compound is a polysulfonated or polysulfated compound.

15            136.    The kit according to claim 135, wherein said polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

137.    The kit according to claim 136, wherein said polysulfonated or polysulfated compound is dextran sulfate.

20            138.    The kit according to claim 137, wherein said dextran sulfate has an average molecular weight of about 5,000 daltons.

139. The method of claim 9, wherein said medium further comprises linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, wherein said buffering salt is N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid] (HEPES), and wherein said sugar is D-glucose.

Add D<sup>3</sup>

Add B<sup>2</sup>

Add C<sup>7</sup>

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